

CLAIMS

What is claimed is:

- 5 1. A method of analyzing a sample for the presence of a member of a specific binding pair,
the method comprising:
 providing a microsphere having an incorporated electroactive marker;
 selecting for the microsphere by formation of a specific binding pair complex; and
 electrochemically testing for the electroactive marker.
- 10 2. The method of claim 1 wherein the microsphere is a polymeric microsphere that is
insoluble in an aqueous solution.
3. The method of claim 2 wherein the microsphere is a polystyrene-based microsphere.
- 15 4. The method of claim 1 wherein the electroactive marker is incorporated into the body of
the microsphere.
5. The method of claim 4, wherein providing comprises incubation of a polymeric
20 microsphere in an organic solvent including an electroactive marker.
6. The method of claim 1 wherein the electroactive marker is incorporated by association
with the surface of microsphere.
- 25 7. The method of claim 1 wherein selecting comprises a first member of the specific
binding pair attached to the microsphere and a second member of the specific binding pair attached to a
substrate.

8. The method of claim 7 wherein the first member of the specific binding pair attached to the microsphere comprises a covalent bond with a functional group on the surface of the microsphere.

9. The method of claim 7 wherein the substrate comprises a magnetic particle.

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10. The method of claim 1 wherein selecting comprises incubation.

11. The method of claim 1 wherein the specific binding pair complex is an antigen/antibody, enzyme/substrate, oligonucleotide/DNA, chelator/metal, enzyme/inhibitor, bacteria/receptor, virus/receptor, hormone/receptor, DNA/RNA, RNA/RNA, or oligonucleotide/RNA complex.

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12. The method of claim 1 further comprising releasing the electroactive marker from the microsphere.

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13. The method of claim 12 wherein releasing comprises solubilizing the microsphere.

14. The method of claim 1 wherein the electroactive marker comprises a metallocene.

15. The method of claim 1 wherein the electroactive marker comprises a nanoparticle.

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16. The method of claim 1 wherein the electroactive marker comprises a metal.

17. The method of claim 1 wherein electrochemically testing comprises measurement of one or more electrical quantities in relationship to one or more chemical parameters.

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18. The method of claim 14 wherein the electrical quantities comprises current, potential or charge.

19. The method of claim 17, wherein measurement of one or more electrical quantities comprises chronopotentiometric detection, stripping potentiometry, stripping chronopotentiometry, anodic stripping voltammetry, cathodic stripping voltammetry, or adsorptive stripping voltammetry.

5 20. A method of analyzing a sample for the presence of two or more analytes, the method comprising:

providing a first microsphere having an incorporated first electroactive marker;

providing a second microsphere having an incorporated second electroactive marker

electrochemically distinguishable from the first electroactive marker;

10 attaching a first binding pair member specific to a first analyte to the first microsphere;

attaching a second binding pair member specific to a second analyte to the second microsphere;

incubating the first microsphere and second microsphere in a solution comprising the sample to be analyzed;

15 selecting for the first microsphere and second microsphere by formation of specific binding pair complexes; and

electrochemically testing for the first electroactive marker and the second electroactive marker.

20 21. The method of claim 20 wherein the microsphere is a polymeric microsphere that is insoluble in an aqueous solution.

22. The method of claim 21 wherein the microsphere is a polystyrene-based microsphere.

23. The method of claim 20 wherein attaching comprises a covalent bond with a functional group on the surface of the microsphere.

25 24. The method of claim 20 wherein the specific binding pair complexes are antigen/antibody, enzyme/substrate, oligonucleotide/DNA, chelator/metal, enzyme/inhibitor,

bacteria/receptor, virus/receptor, hormone/receptor, DNA/RNA, RNA/RNA, or oligonucleotide/RNA complexes.

25. The method of claim 20 further comprising releasing the first electroactive marker from
5 the first microsphere and the second electroactive marker from the second microsphere.

26. The method of claim 25 wherein releasing comprises solubilizing the first microsphere
and the second microsphere.

10 27. The method of claim 20 wherein the first electroactive marker and the second
electroactive marker comprise metallocenes.

28. The method of claim 20 wherein the first electroactive marker and the second
electroactive marker comprise nanoparticles.

15 29. The method of claim 20 wherein the first electroactive marker and the second
electroactive marker comprise metal.

30. The method of claim 20 wherein electrochemically testing comprises measurement of
20 one or more electrical quantities in relationship to one or more chemical parameters.

31. The method of claim 30 wherein the electrical quantities comprises current, potential or
charge.

25 32. The method of claim 30, wherein measurement of one or more electrical quantities
comprises chronopotentiometric detection, stripping potentiometry, stripping chronopotentiometry,
anodic stripping voltammetry, cathodic stripping voltammetry, or adsorptive stripping voltammetry.

33. A microsphere for electrochemical detection of a member of a specific binding pair, comprising a polymeric microsphere having an organic solvent soluble hydrophobic electroactive marker incorporated into the body of the microsphere and at least one functional group on the surface of the
5 microsphere.

34. The microsphere of claim 33 wherein the soluble hydrophobic electroactive marker is non-magnetic.

10 35. The microsphere of claim 34 wherein the soluble hydrophobic electroactive marker is a metallocene.

36. The microsphere of claim 35 wherein the metallocene is ferrocene or ferrocenecarboxaldehyde.

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37. The microsphere of claim 33 wherein the at least one functional group is a sulfate surface group, aldehyde group, aliphatic amine group, amide group, aromatic amine group, carboxylic acid group, chloromethyl group, epoxy group, hydrazide group, hydroxyl group, sulfonate group or tosyl group.

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38. The microsphere of claim 33 wherein the polymeric microsphere is a polystyrene-based microsphere.

39. The microsphere of claim 38 wherein the polystyrene-based microsphere has a diameter
25 between about 0.01 μm and about 100.0 μm .

40. The microsphere of claim 38 wherein the polystyrene-based microsphere has a diameter between about 0.3 μm and about 20 μm .